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A New Validated Reverse Phase Liquid Chromatography Method for the Simultaneous Estimation of Sulfadoxine and Pyrimethamine in Combined Tablet Dosage Form

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ABSTRACT
A reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Sulfadoxine and Pyrimethamine in pharmaceutical dosage forms. The method optimization involved systematic trials with varying chromatographic conditions, including column selection, mobile phase composition, pH, flow rate, and detection wavelength. The final method employed a Platisil C18-ODS column (250×4.6 mm, 5 µm) with a mobile phase of 50% 0.1% formic acid (pH 4.5) and 50% methanol, at a flow rate of 1.0 ml/min, and UV detection at 230 nm. System suitability parameters such as retention time, theoretical plates, and tailing factor were within acceptable limits, confirming the method's efficiency and reliability. Validation was performed according to ICH Q2(R1) guidelines, demonstrating excellent analytical performance. Assay results showed drug content of 99.1% for Sulfadoxine and 98.6% for Pyrimethamine. Linearity was observed over wide concentration ranges with correlation coefficients of 0.9997 and 0.9996, respectively. Precision and intermediate precision studies yielded %RSD values below 2%, indicating high repeatability and ruggedness. Recovery studies confirmed accuracy within the 98–102% range, while LOD and LOQ values (0.02 µg/ml for Sulfadoxine and 0.05 µg/ml for Pyrimethamine) highlighted the method's sensitivity. Robustness testing under slight variations in flow rate and mobile phase composition showed consistent results, affirming the method's suitability for routine quality control of combined dosage formulations.
Keywords: RP-HPLC, Sulfadoxine, Pyrimethamine, ICH Q2(R1), system suitability, robustness

INTRODUCTION

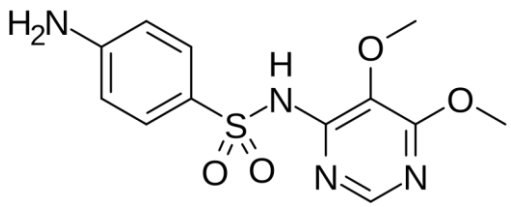

Fig.1: Sulfadoxine Drug Profile

Sulfadoxine:

- IUPAC Name: 4-Amino-N-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide1
- Molecular Formula: $C_{12}H_{14}N_4O_4S$1
- Molecular Weight: 310.33 g/mol1
- Melting Point: 190 to 194 °C1
- pKa: Approximately 5.62
- Category: Sulfonamide antibiotic
- Solubility: Poorly soluble in water

Description
Sulfadoxine is a long-acting sulfonamide used primarily in combination with pyrimethamine to treat or prevent malaria21. It is also used for respiratory and urinary tract infections2.

Mechanism of Action
Sulfadoxine inhibits the enzyme dihydropteroate synthetase, which is essential for the synthesis of folic acid in Plasmodium falciparum. This inhibition disrupts DNA synthesis and cell growth in the parasite.

Pharmacodynamics
Sulfadoxine helps inhibit the enzyme dihydropteroate synthetase, which is necessary for the conversion of PABA to folic acid. This inhibition is crucial for the parasite's reproduction2.

Pharmacokinetics

- Absorption: Well absorbed from the gastrointestinal tract
- Distribution: Widely distributed in body tissues
- Metabolism: Metabolized in the liver
- Route of Elimination: Primarily excreted in the urine
- Protein Binding: Approximately 90%
- Half-Life: Around 7 to 9 days

Primary Use: Treatment and prevention of malaria, especially in combination with pyrimethamine

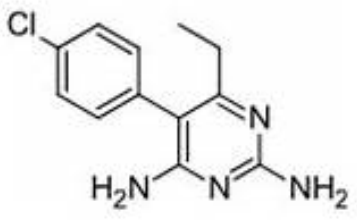

Fig.2: Pyrimethamine Drug Profile

Pyrimethamine:

- IUPAC Name: 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine1
- Molecular Formula: $C_{12}H_{13}ClN_4$1
- Molecular Weight: 248.71 g/mol1
- Melting Point: 233 to 234 °C1
- pKa: Approximately 7.32
- Category: Antiparasitic2
- Solubility: Poorly soluble in water

Description: Pyrimethamine is an antiparasitic medication used to treat and prevent malaria and toxoplasmosis. It is often used in combination with a sulfonamide

Mechanism of Action

Pyrimethamine inhibits the enzyme dihydrofolate reductase, which is essential for the synthesis of folic acid in parasites. This inhibition disrupts DNA synthesis and cell growth in the parasite.

Pharmacodynamics

Pyrimethamine works by inhibiting folic acid metabolism, which is crucial for the parasite's reproduction

Pharmacokinetics

- Absorption: Well absorbed from the gastrointestinal tract
- Distribution: Widely distributed in body tissues.
- Metabolism: Metabolized in the liver
- Route of Elimination: Primarily excreted in the urine
- Protein Binding: Approximately 87%
- Half-Life: Around 96 hours

METHODOLOGY

Table 1: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Sulfadoxine and Pyrimethamine	Supplied by MSN LAB
2	Formic acid	FINAR chemical LTD
3	Water and Methanol for HPLC	Standard solutions Ltd
4	Acetonitrile for HPLC	Standard solutions Ltd
5	HCl, H_2O_2, NaOH	MERCK

Wave length selection:

UV spectrum of 10µg/ml Sulfadoxine and Pyrimethamine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm and the isobestic λmax of both the drugs obtained at 230 nm.

Optimization of Column:

Platisil C_{18}-ODS, (250×4.6mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions

Instrument used : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector
Temperature : Ambient
Column : Platisil C_{18}-ODS, (250×4.6mm, 5µm)
Buffer : 0.1% Formic acid (Ph 4.5)
Mobile phase : 50% 0.1% Formic acid: 50% Methanol
Flow rate : 1.0ml per min
Wavelength : 230nm
Injection volume : 10µl
Run time : 10min.

Preparation of buffer and mobile phase:

Preparation of 0.1% Formic acid PH 4.5:

To prepare 0.1% Formic acid buffer solution by adding 1ml of Formic acid in 100oml HPLC water adjust the solution to PH 4.5 by using NAOH solution.

Preparation of mobile phase:

Mix a mixture of above 0.1% Formic acid (PH 4.5) 500ml (50%) and 500 ml MEOH (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

0.1% Formic acid (PH 4.5): MEOH (50:50ml)

System Suitability: Tailing factor for the peaks due to Sulfadoxine and Pyrimethamine in Standard solution should not be more than 2.0

Theoretical plates for the Sulfadoxine and Pyrimethamine peaks in Standard solution should not be less than 2000

Calculation: (For Sulfadoxine and Pyrimethamine)

$$\%\,Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT= average area counts of sample preparation.
AS= average area counts of standard preparation.
WS= Weight of working standard taken in mg.
P= Percentage purity of working standard
LC=Label Claim mg/ml.

Acceptance criteria of System Suitability:

Tailing factor should be less than 2
Theoretical Plates should be above 2000

Method validation parameters:

ASSAY:

Standard Solution Preparation: Accurately weigh and transfer 20 mg of Sulfadoxine and 12mg Pyrimethamine working standard into a 20 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.(30ppm, 18ppm).

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 20 mg of Sulfadoxine and 12mg Pyrimethamine equivalent weight of the sample into a 20 ml clean dry volumetric flask add about 7ml of Diluents and sonicate to Dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject 30 µL of the standard, sample into the chromatographic system and measure the areas for the Sulfadoxine and Pyrimethamine peaks and calculate the % Assay by using the formulae.

RESULTS

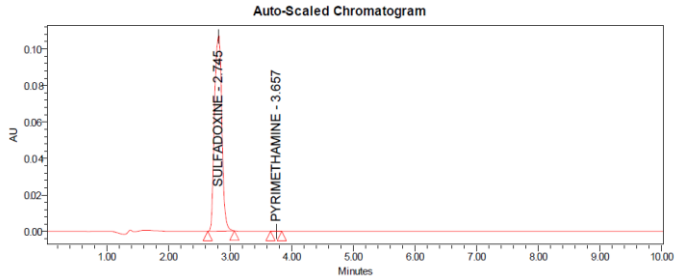

Fig.3: Chromatogram for system suitability

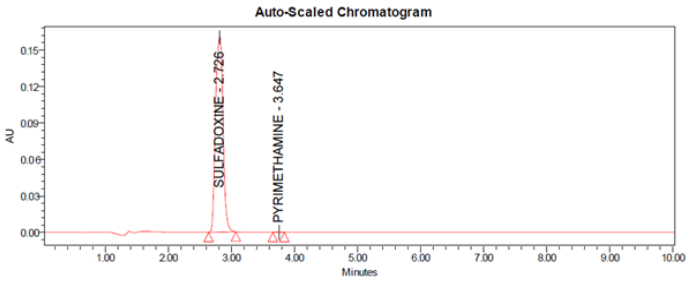

Fig.4: Chromatogram for Sample

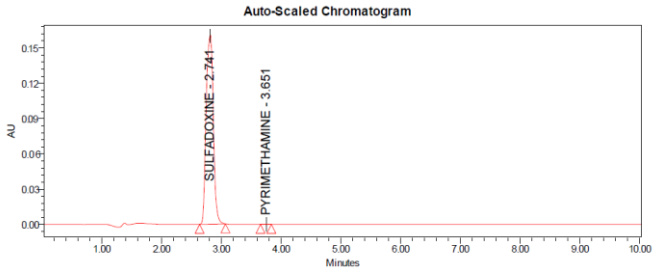

Fig.5: Chromatogram for Standard

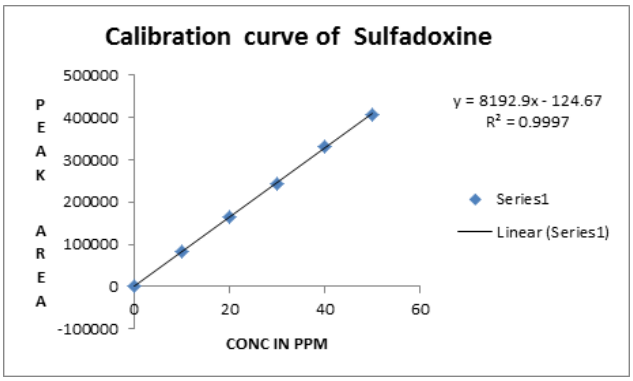

Fig.6: Calibration graph for Sulfadoxine

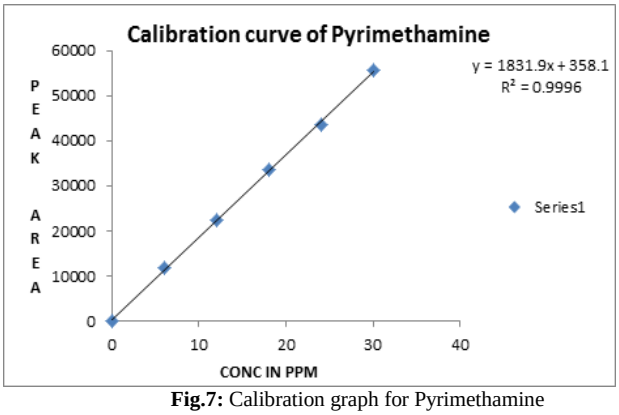

Fig.7: Calibration graph for Pyrimethamine

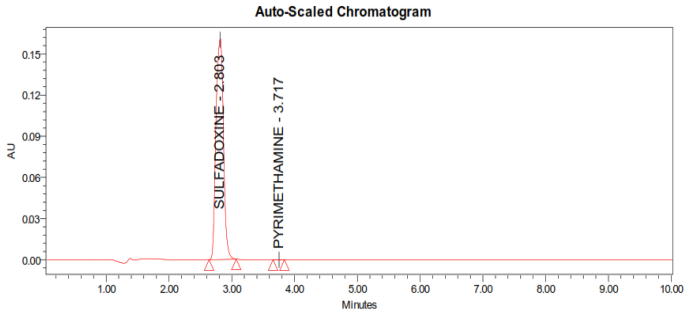

Fig.8: Chromatogram for ID Precision -6

Table 3: Results of Intermediate precision for Sulfadoxine and Pyrimethamine

Injection	Area of Sulfadoxine	Area of Pyrimethamine
Injection-1	250623	34521
Injection-2	254445	34158
Injection-3	253728	34081
Injection-4	255957	34220
Injection-5	252247	34989
Injection-6	251901	34654
Average	253150.2	34437.17
Standard Deviation	1931.398	318.9866
%RSD	0.8	0.9

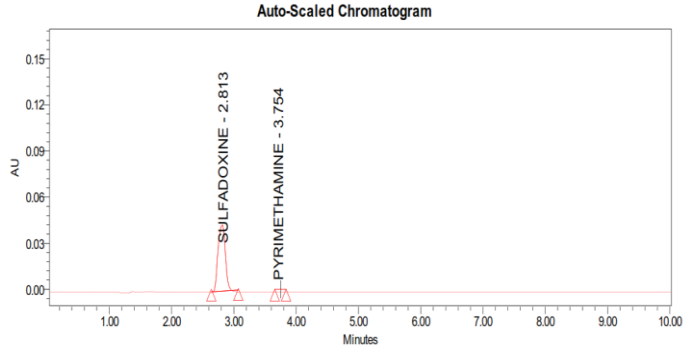

Fig.9: Chromatogram of Sulfadoxine and Pyrimethamine showing LOD

Table 4: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc.
Sulfadoxine	57	162	2.84	0.02µg/ml
Pyrimethamine	57	167	2.93	0.05µg/ml

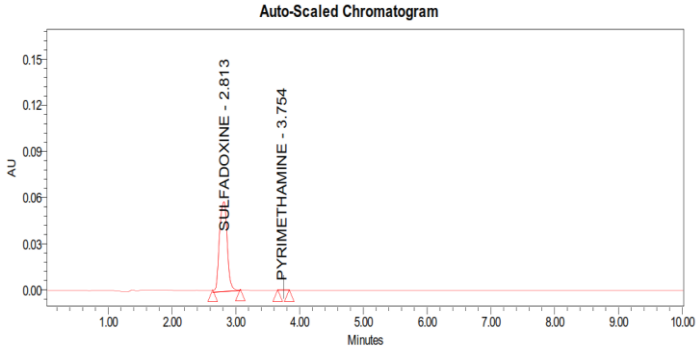

Fig.10: Chromatogram of Sulfadoxine and Pyrimethamine showing LOQ

Table 5: Results of LOQ

Drug name	Baseline noise(µV)	Signal Obtained(µV)	S/N ratio	Conc.
Sulfadoxine	57	550	9.65	0.06µg/ml
Pyrimethamine	57	565	9.91	0.17µg/ml

Table 6: Accuracy (recovery) data for Sulfadoxine

% Concentration	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	122758	10	10.0	99.7	99.6
100%	244258	20	19.8	99.2	
150%	369851	30	30.0	100.1	

Table 7: Accuracy (recovery) data for Pyrimethamine

% Concentration	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	16558	6	5.92	98.7	98.8
100%	32987	12	11.8	98.3	
150%	49992	18	17.9	99.4	

Table 8: Results for variation in flow for Sulfadoxine and Pyrimethamine

S. No	Flow Rate(ml/min)	System Suitability Results Sulfadoxine	
		USP Plate Count	USP Tailing
1	0.8	7541	1.02
2	1	7584	1.10
3	1.2	7514	1.01

Table 9: Results for variation in mobile phase composition for Sulfadoxine and Pyrimethamine

S. No	Change in OrganicComposition in theMobile Phase	System Suitability Results Pyrimethamine	
		USP Plate Count	USP Tailing
1	20% less(40ml)	3568	1.03
2	*Actual(50ml)	3555	1.24
3	20% more(60ml)	3589	1.12

CONCLUSION

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Sulfadoxine and Pyrimethamine in pharmaceutical dosage forms. Multiple trials were performed by optimizing various chromatographic conditions including column types, mobile phase composition, pH, flow rate, and detection wavelength. The finalized and optimized method utilized a Platisil C18-ODS column (250×4.6 mm, 5 µm) with a mobile phase consisting of 50% 0.1% formic acid (pH 4.5) and 50% methanol**, a** flow rate of 1.0 ml/min**,** and detection at 230 nm**.** The system suitability parameters such as retention time**,** theoretical plates**,** and tailing factor were within the acceptable limits, confirming the effectiveness of the developed method. The validated method met all ICH Q2(R1) guidelines for analytical performance. Assay results showed high accuracy, with content found to be 99.1% for Sulfadoxine and 98.6% for Pyrimethamine. The method was proven to be linear over a wide concentration range with correlation coefficients of 0.9997 for Sulfadoxine and 0.9996 for Pyrimethamine. Precision and intermediate precision showed %RSD values below 2, indicating excellent repeatability and ruggedness. The accuracy/ recovery studies yielded mean recoveries within the acceptable range (98–102%). LOD, LOQ values demonstrated the sensitivity of the method, with limits as low as 0.02 µg/ml for Sulfadoxine and 0.05 µg/ml for Pyrimethamine. Moreover, robustness studies revealed the method's stability under slight variations in flow rate and mobile phase composition. Overall, the method is simple, reliable, precise, and suitable for routine quality control analysis of Sulfadoxine and Pyrimethamine in combined dosage formulations.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not for profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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